A STUDY OF THE ERROR IN SUGAR DETERMINATIONS CAUSED BY SULFONAMIDE DRUGS AND THE CHARACTERIZATION OF THE COMPOUNDS RESPONSIBLE FOR THE INTERFERENCE

by

MARY CLAY DODSON

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INTRODUCTION

This work began as a result of the observation that the sugar level of beef blood containing sulfanilamide remained much higher than would normally be expected in the absence of the drug after several days in the ice box. The sulfonamide drug had been added to prevent bacterial growth. In order to try to explain this phenomenon, a fresh sample of beef blood was obtained. Part of this was kept as a control, and part had sulfanilamide added. Blood sugar determinations were made immediately, and it was found that the sugar level of the blood containing sulfanilamide was apparently lower than that of the control. It stands to reason that this difference was apparent and not real. Since this observation could be repeated at will, it became obvious that the presence of sulfanilamide in blood introduced an error in glucose determinations when run by the Shaffer-Hartmann method.

Because of the wide use of sulfonamide drugs, it was felt that an investigation of this phenomenon would be advisable.

PART I

INTERFERENCE OF SULFONAMIDE DRUGS IN SUGAR DETERMINATIONS

EXPERIMENTAL

The first problem was to find a method for running quantitative sugar determinations in the presence of sulfanilamide since it was shown that the Shaffer-Hartmann and other alkaline copper methods were not accurate under such conditions (1).

Somogyi (2) recently published a urine sugar method involving no copper salts in the reagents. It has been demonstrated that the presence of sulfanilamide does not cause an error when this method is used and consequently it was adopted throughout this work to establish the sugar level of urine and other solutions employed. Because of its excellence and simplicity, the Somogyi method probably will become widely used in clinical laboratory work for quantitative urine sugar estimations.

The method as adapted is as follows: In an 8" x 1" test tube, 10 ml. of 10 per cent sodium carbonate are placed and 1 ml. of urine added. The mixture is heated for 8 minutes in a boiling water bath. After cooling, the color produced is read in the photoelectric colorimeter and the amount of glucose calculated from a standard curve prepared in the following manner: Glucose solutions containing 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 gms. per cent are prepared. To 1 ml. of each of these solutions 10 ml. of 10 per cent sodium carbonate are added. After 8 minutes in a boiling water bath, the color developed is measured in a Klett photoelectric colorimeter and the readings plotted against the concentrations. It was found that a straight line curve resulted indicating that within the limits of concentrations employed, there is

good observance to Beer's law. The standard curve used in this work is shown in Graph I.

Reducing sugars in the presence of alkali have the property of coloring the solution yellow on heating, although the chemical nature of these reactions are not understood. The intensity of the yellow color is proportional to the amount of glucose present under the conditions of the method.

This method was checked against the Shaffer-Hartmann method using (a) untreated urine, (b) urine after yeast fermentation, and (c) iron filtrates (3) of urine. Table I shows typical data obtained from such determinations.

TABLE I

Series of urine sugars showing comparison of values obtained with the Somogyi and Shaffer-Hartmann methods.

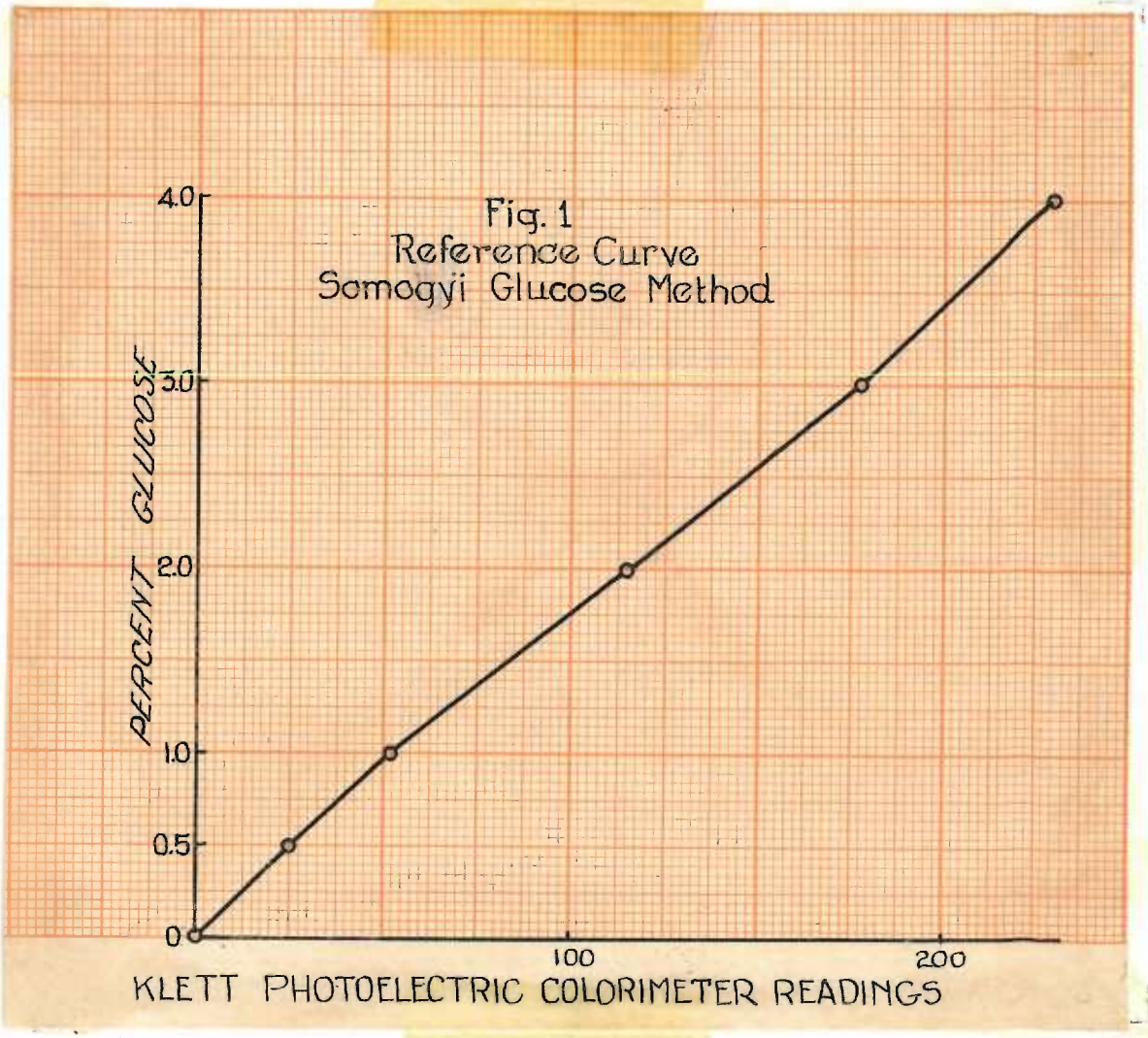
Shaffer-Hartmann untreated urine Glucose gms. per 100 ml.	Shaffer-Hartmann yeast fermentation Glucose gms. per 100 ml.	Shaffer-Hartmann iron filtrate Glucose gms. per 100 ml.	Somogyi untreated urine Glucose gms. per 100 ml.
0.378	0.366	0.382	0.34
0.403	0.383	0.396	0.56 *
0.480	0.356	0.388	0.58 *
0.946	0.751	0.792	0.86
1.04	0.886	0.919	0.88
1.46	1.36	1.39	1.48
1.50	1.30	1.43	1.20
3.93	2.53	3.40	3.10

* It was later found advisable to pretreat urine with a small amount of Norite to remove the chromogenic substances present. This was especially important in the case of urines with low sugar levels because the yellow color of the urine was sufficient to impart a significant fraction of the total color formed. After adopting this procedure, better agreement was obtained.

GRAPH I

Reference Curve
Somogyi Glucose Method

Fig. 1
Reference Curve
Somogyi Glucose Method



Since the results of the Somogyi method agreed so well with the true sugar level (yeast fermentation) it was felt that it constituted an adequate method for determinations in this work.

QUANTITATIVE GLUCOSE DETERMINATIONS IN AQUEOUS SOLUTIONS CONTAINING SULFONAMIDE DRUGS.

It seemed advisable to test the effect of sulfanilamide as well as the other clinically employed sulfonamide drugs for their capacity to interfere in sugar determinations. This was first done using aqueous solutions of the drug and sugar, in order to determine the amount of interference and a possible explanation for its mechanism.

Various methods employing alkaline copper reagents were studied. Following is a typical experiment which indicates the approach to this problem. A 1 per cent glucose solution was made and sulfanilamide added to yield concentrations of from 25 mgs. per cent to 200 mgs. per cent. Sugar determinations were run on these solutions by the Shaffer-Hartmann method. It was found that as the amount of sulfanilamide increased, the percentage recovery decreased. In a glucose solution containing 200 mgs. per cent sulfanilamide which had actually only 0.2 mg. of the drug in 5 ml. of the 1 to 50 dilution used for the determination, an 8 per cent error was found. Such an experiment was repeated using various concentrations of glucose. The degree of interference was found to be primarily dependent on sulfanilamide concentration and not on sugar concentration. Table II shows this clearly.

TABLE II

Data showing error caused by various amounts of sulfanilamide in quantitative sugars determined by the Shaffer-Hartmann method.

Sulfanilamide mgs. per 100 ml.	Glucose gms. per. 100 ml. Present	Determined	Sulfanilamide present in aliquot used for analysis mgs.	Per cent Recovery
0	0.99	0.99	0.00	100.0
25	0.99	0.99	0.025	100.0
50	0.99	0.958	0.05	96.9
150	0.99	0.936	0.15	94.5
200	0.99	0.915	0.20	92.4
0	0.48	0.48	0.00	100.0
100	0.48	0.452	0.10	94.1
200	0.48	0.427	0.20	89.1
300	0.48	0.410	0.30	85.4

Sulfathiazole and sodium sulfapyridine were then tested under similar experimental conditions. The results will be found in Table III. It is significant that the direction of error caused by either of these drugs is opposite to that found in the case of sulfanilamide. Also the error is considerably less when comparable amounts of the drug are employed. An attempt to explain this unexpected result will be found in the discussion.

TABLE III

Data showing error caused in quantitative sugar determinations by sulfathiazole and sulfapyridine.

Sulfathiazole mgs. per 100 ml.	Glucose gms. per. 100 ml. Present	Determined	Sulfathiazole present in aliquot used for analysis mgs.	Per cent Recovery
0	0.480	0.480	0.0	100
100	0.480	0.497	0.1	102
200	0.480	0.509	0.2	105
300	0.480	0.523	0.3	108
Sulfapyridine mgs. per. 100 ml.				
0	0.509	0.509	0.0	100
100	0.509	0.537	0.1	105
200	0.509	0.548	0.2	108
300	0.509	0.548	0.3	108

QUANTITATIVE DETERMINATIONS IN BLOOD.

Attention was next turned to biological fluids to determine the nature and extent of interference caused by sulfanilamide in sugar determinations. Since patients, who are kept on sulfanilamide therapy, seldom ever have higher blood levels than 25 mgs. per cent, it was felt that this would be the highest value requiring investigation. To 100 ml. aliquot of a blood sample 25 mgs. sulfanilamide were added and blood sugars were run by the Shaffer-Hartmann method on this sample and also on another aliquot containing no sulfanilamide. The sugar level of the control was found to be 90 mgs. per cent and that of the experimental sample 85 mgs. per cent. This is a 94.4 per cent recovery and it is interesting to note that the aliquot of the blood filtrate used for analysis contained 0.125 mg. sulfanilamide. This error is in close agreement with that found in pure solutions containing a comparable

amount of sulfanilamide. See Table II. A 94.1 per cent recovery was found when the aliquot of pure solution used for analysis contained 0.10 mg. of sulfanilamide. In clinical work an error such as this is of little consequence but for meticulous results it must be given consideration.

QUANTITATIVE DETERMINATIONS IN URINE.

Because urine from patients on sulfanilamide therapy contains far more of this drug than does their blood, attention was turned to a study of the error involved in urine sugar determinations. To a urine sample containing 0.79 per cent glucose, sulfanilamide was added to give a final concentration of 300 mgm. per cent. The glucose level then had an apparent value of 0.72 per cent which represents a 91 per cent recovery. This degree of interference is in good agreement with that found in the work with blood and with pure solutions containing comparable amounts of the sulfonamide drug.

In general the greatest interference was found when the glucose values were low. One urine sample which contained 0.416 gm. glucose per 100 ml., showed on the addition of sulfanilamide to yield concentrations of 150 mgs. per cent and 300 mgs. per cent, only 88 per cent and 76 per cent recovery respectively. This error is considerably greater than any encountered in the work on pure solutions even when the levels of sugar and sulfanilamide were comparable. No explanation for this is at hand.

DISCUSSION

It was demonstrated early in this work (1) that the interference of sulfanilamide in sugar determinations by methods employing alkaline copper reagents was due to the formation of a cuprous-copper sulfanilamide complex. This removes a certain amount of cuprous ion and thus less remains for oxidation by iodine and consequently the results are

erroneously low.

It is possible to make cuprous-copper sulfanilamide complex by boiling 5 ml. of an aqueous solution containing 100 mgs. per cent glucose and 400 mgs. per cent sulfanilamide with 5 ml. Shaffer-Hartmann reagent. No cuprous oxide appears. Instead a white crystalline material precipitates on cooling. This is composed of cuprous-copper and sulfanilamide. The concentration of sulfanilamide mentioned above is, of course, much higher than would ordinarily be found in sugar determinations in biological fluids because of the large dilution factor. It is felt that the mechanism of such crystal formation offers a possible explanation for the interference in sugar determinations.

This theory was further borne out when sulfanilamide copper complex was prepared and purified and then added to Shaffer-Hartmann reagent in varying amounts. In each case the actual titration difference agreed quite closely with the theoretical titration difference. This figure was computed by finding the theoretical amount of cuprous-copper (I) in the sample of the complex used, that would be made available for the reaction with iodine on acidification. Then knowing that 0.318 mg. cuprous-copper is equivalent to 1 ml. of 0.005 N. sodium thiosulfate it is possible to compute the theoretical titration difference.

In each case, however, the actual titration difference was a little lower than the theoretical titration difference. This is interpreted to mean that on acidifcation most of the complex but not quite all is decomposed with the liberation of most of the cuprous ion. The difference between the theoretical and actual titration values are always just about the same regardless of the amount of sulfanilamide copper complex indicating that a more or less constant amount of the complex is not decomposed under these conditions of acidification.

Table IV indicates these points.

TABLE IV

Comparison of actual and theoretical titration differences due to cuprous ion liberated from copper sulfanilamide complex with Shaffer-Hartmann reagent.

Sulfanilamide Complex present in aliquot used for analysis	Theoretical Titration Difference	Actual Titration Difference
mgs.	ml.	ml.
5.0	5.30	5.15
3.0	3.36	3.15
1.5	1.67	1.45
0.75	0.83	0.50
0.375	0.42	0.12

From these results it is possible to place the following interpretation on the overall picture. On heating glucose in an alkaline copper solution some of the cupric ion is reduced to cuprous ion. When sulfanilamide is present the cuprous ion is taken up to form a copper sulfanilamide complex. On acidification, however, the greater part of this complex, but not quite all, is decomposed liberating cuprous ion which can then react with the iodine. The amount of cuprous ion that remains unavailable for oxidation by iodine is closely correlated with errors found in sugar determinations.

At the present time there is no adequate explanation for the action of sulfapyridine and sulfathiazole. When 10 mgs. of sulfanilamide are added to 5 ml. Shaffer-Hartmann reagent, practically no titration difference compared to normal blanks is found. With sulfathiazole, however, under the same conditions over 2 ml. titration difference is obtained. This actually represents a gross error. It would seem, therefore, that this interference is not related to the formation of a

cuprous copper complex with sulfathiazole since similar titration differences are found with sulfathiazole in the presence of sugar and this error is in a direction opposite to that found when sugar is determined in the presence of sulfanilamide.

QUALITATIVE URINE SUGARS.

The effects of sulfanilamide and various other sulfonamide drugs were investigated in qualitative urine sugar tests employing various of the alkaline copper methods. Those used most widely in clinical laboratory work are the Benedict test, the Clinitest, (Effervescent Product, Inc.), and the Sheftel test (Eli Lilly and Company). It was thought advisable to start with sugar free urine and add varying amounts of glucose and of the sulfonamide drug under test in order to control both of these variables.

The Sheftel test was included in this work but practically no interference was observed from any of the drugs tested; consequently such data are omitted from the table below. Such observations remain unexplained, since the reagents employed in this method are similar to those of the Clinitest in many respects. While there was no interference from the sulfonamide drugs, the accuracy of the method was not found to be as great as that of the Clinitest and Benedict methods.

In Table V data will be found indicating the degree of interference due to various levels of different sulfonamide drugs with several qualitative urine sugar methods.

TABLE V

Interference caused by several sulfonamide drugs in some of the commonly employed qualitative urine sugar tests.

Glucose gms. per 100 ml.	Sulfanilamide mgs. per 100 ml.	Clinitest Reading	Benedict Reading	Somogyi gms. per 100 ml.
0.50	0	+	+	0.53
	100	trace	trace	0.53
	200	0	trace	0.53
	400	0	trace	0.53
0.75	0	+ +	+ +	0.74
	300	+	+	0.74
1.0	0	+ + +	+ + +	1.00
	300	+ +	+ +	1.02
2.0	0	+ + + +	+ + + +	1.86
	150	+ + + +	+ + +	1.86
	300	+ + + +	+ + +	1.86
	Sulfapyridine mgs. per 100 ml.			
0.50	0	+	+	0.56
	150	trace	+	0.56
0.75	0	+ +	+ +	0.73
	150	+ +	+ +	0.75
1.0	0	+ + +	+ + +	1.08
	150	+ + +	+ + +	1.08

Glucose gms. per 100 ml.	Sulfadiazene mgs. per 100 ml.	Clinitest Reading	Benedict Reading	Somogyi gms. per 100 ml.
0.50	0	+	+	0.56
	100	+	+	0.58
0.75	0	+ +	+ +	0.76
	100	+ +	+ +	0.76
1.0	0	+ + +	+ + +	1.08
	100	+ + +	+ + +	1.06
	Mono-methyl Sulfadiazene mgs. per 100 ml.			
0.50	0	+	+	0.54
	100	+	+	0.54
0.75	0	+ +	+ +	0.68
	100	+ +	+ +	0.68
	Sulfathiazole mgs. per 100 ml.			
0.50	0	+	+	not determined
	200	+	+	" "
0.75	0	+ +	+ +	" "
	225	+ +	+ +	" "

With sulfanilamide there is a significant interference especially in the urine with low sugar values. A urine reading one + with Benedict solution showed no reduction after the addition of sulfanilamide to yield a concentration of 200 mgs. per cent. In almost every case, as the amount of sugar increased the Benedict and Clinitest were read just one + lower than a similar urine sample devoid of sulfanilamide. The mechanism for this interference is thought to be the same as that proposed in the quantitative test with the exception that the crystals formed are not decomposed, as no acidification takes place in the qualitative tests.

All of the other sulfonamide drugs tested, showed no significant error although there was a tendency in the case of sulfathiazole to upgrade the results but never as much as one +. It is felt that this is due to the fact that the sulfathiazole copper complex formed, is orange under these conditions of alkalinity. This tends to make the results appear higher, whereas with sulfanilamide the tannish-white complex formed, tends in the Benedict and Clinitest to give a greenish cast to the solution, thus making the results appear lower. The fact that part of the cuprous ion is removed as a soluble complex, colorless in solution, thus yielding less cuprous oxide, is an additional mechanism to account for the low readings. Qualitative urine sugars are graded both on the color of the supernatant fluid and the amount and color of the cuprous oxide formed.

It was felt that the interference of sulfanilamide in qualitative urine sugar determinations would be especially important in the case of diabetics on sulfanilamide therapy. For this reason a series of diabetic urines were obtained and varying amounts of sulfanilamide added and qualitative sugar tests carried out. Here, again, the results showed a similar interference. The following table illustrates these findings.

TABLE VI

The interference caused by sulfanilamide in the determination of sugar in diabetic urine.

Glucose (Somogyi) gms. per 100 ml.	Sulfanilamide mgs. per 100 ml.	Benedict Reading	Clinitest Reading
0.47	0	+	trace
	150	trace	-
	300	trace	-

Glucose (Somogyi) mgs. per 100 ml.	Sulfanilamide mgs. per 100 ml.	Benedict Reading	Clinitest Reading
0.66	0	+ +	+ +
	150	+	+
	300	+	+
1.38	0	+ + +	+ + +
	150	+ + +	+ +
	300	+ + +	+ +
1.86	0	+ + + +	+ + + +
	150	+ + +	+ + + +
	300	+ + +	+ + + +
2.92	0	+ + + +	+ + + +
	150	+ + + +	+ + +
	300	+ + + +	+ + +
3.20	0	+ + + +	+ + + +
	150	+ + + +	+ + + +

Results of qualitative urine sugars determined on urine of patients under sulfanilamide therapy must be viewed with skepticism if such tests are made with the Clinitest or Benedict method. The clinical significance of these findings, however, assumes less importance at the moment because of the decreased use of sulfanilamide and the increased use of derivatives of this compound.

If a qualitative sugar determination must be made on a sample containing high levels of sulfanilamide, the Somogyi method is recommended. The interference can also be obviated by removing the drug before the test is carried out. This can be accomplished as follows: To 10 ml. of urine are added about 0.5 gm. of norite to adsorb the sulfanilamide. After shaking about half a minute the sample is

filtered. Urine treated in this manner no longer contains sulfanilamide as tested by the Bratton and Marshall technique (5). Sugar determinations by the Somogyi method before and after the norite treatment indicated that no sugar was lost through adsorption.

Urines pretreated in this way show correct readings with the Clinitest method. With the Benedict test, however, the results are hard to grade as urine clarified in this manner gives atypical colors just as pure glucose solutions do.

PART II

PREPARATION, ISOLATION AND CHARACTERIZATION OF CUPROUS-COPPER COMPLEXES OF SOME SULFONAMIDE DRUGS.

PREPARATION

Since sulfanilamide with its marvelous chemotherapeutics properties was given to the world in 1935, the chemist has synthesized thousands of derivatives in the hopes of finding even more effective drugs. At the present time, there are perhaps ten of these compounds that are widely used in the field of medicine. A review of the literature revealed no reports of copper sulfonamide complexes similar to those first isolated in this work (4). Therefore, various of these compounds were prepared for analysis.

Copper sulfonamide drug complexes can be prepared in an alkaline copper solution containing glucose which reduces cupric ion to cuprous ion. It is possible under the right conditions of temperature and concentration to form these crystalline complexes with a number of the sulfonamide drugs.

Sulfanilamide, sulfathiazole and sulfapyridine copper complexes can be prepared in the following manner: To 250 ml. of Shaffer-Hartmann reagent No. 50, 100 ml. of an aqueous solution containing 1 gm. sulfonamide drug and 150 mgs. glucose are added. This mixture is slowly heated over a flame with frequent shaking until the complex precipitates. The crystals are then removed by filtration, purified by washing several times in cold water, and dried in a vacuum dessicator.

With sulfadiazene, monomethyl sulfadiazene and dimethyl sulfadiazene, it was found necessary to change the proportion of glucose and sulfonamide drug in order to avoid the formation of cuprous oxide. With

these drugs the following procedure is followed: To 250 ml. Shaffer-Hartmann reagent No. 50, 250 ml. water containing 80 mgs. glucose and 1.25 gm. sulfonamide drug are added. Using these proportions, cuprous ion was formed at a slow enough rate for the sulfonamide drug to react with it and form the complex without the precipitation of cuprous oxide. After the crystals are filtered it is possible to obtain a second crop by adding another 80 mgs. of glucose and reheating the solution cautiously.

To date it has not been possible to form sulfaguanidine copper complex under any conditions of temperature and concentration employed.

CHEMICAL AND PHYSICAL PROPERTIES

These complexes are insoluble in water but soluble in dilute acid and alkali. It is interesting to note that sulfanilamide copper complex decomposes readily in dilute acid with the liberation of cuprous oxide; the other complexes require a much higher concentration of acid. The melting points could not be obtained on any of the compounds as decomposition begins between 200-300°C.

Sulfanilamide copper complex is the only one of the group prepared that discolors on standing. The other crystals retain their original color. Sulfathiazole forms a white complex under the above conditions of temperature and concentration. On increasing the alkalinity, however, the complex formed is orange in color, but the various colored crystals appear identical microscopically. Sulfapyridine, and dimethyl sulfadiazine also form white complexes, whereas sulfadiazene and monomethyl sulfadiazene form yellow complexes.

ANALYSES

For the sulfur analysis a modification of the Liebig Alkali Method (6) was used. In a silver crucible, 3.6 gm. of KOH and 0.33 gm. of KNO_3 are fused over an electric hot plate and then allowed to cool. To this a weighed sample of the purified crystals is added and the mixture heated until oxidation is complete. Caution must be exercised at the beginning of the heating to avoid excess foaming. After cooling, the residue is transferred to a 250 ml. beaker. The black precipitate of cupric oxide is filtered off and the filter paper washed with hot water. The filtrate is neutralized with concentrated HCl to the phenolthalein endpoint and 1 ml. in excess added. The solution is then heated to boiling and 10 ml. 10 per cent $BaCl_2$ added dropwise. Heat is applied for about one half hour longer to allow the $BaSO_4$ crystals to aggregate. After standing overnight the $BaSO_4$ is filtered into a weighed Gooch crucible and washed with hot distilled water until all chloride ion is removed. The crucible is heated in an oven at 110°C to constant weight. The amount of sulfur present is calculated from the weight of $BaSO_4$ and the percentage in the sample thus determined.

The following method (7) was used for the determination of copper. A weighed sample of crystals is placed in a 12" x 1" pyrex tube and 5 ml. H_2O and 5 ml. concentrated H_2SO_4 are added. This is heated to charring, allowed to cool and then 0.2 ml. concentrated HNO_3 added. This is repeated until the solution is clear indicating complete oxidation. To remove the HNO_3 completely it is necessary to add water and boil it off several times. Finally the sample is diluted with water and transferred to a 100 ml. volumetric flask and diluted to volume. A 10 ml. aliquot is pipetted into 125 ml. Erlenmeyer flask. Concentrated NH_4OH

is added until the maximum blue color of the cupric ammonium complex is formed. Glacial CH_3COOH is next added until the disappearance of the blue color and then 1 ml. in excess. After 2 gm. KI are added the solution is titrated with 0.01 N sodium thiosulfate.* Just before the endpoint is reached 1 ml. of a 1% starch solution is added and the titration completed.

The nitrogen determinations were done in the following manner: A weighed sample of crystals is placed in a 12" x 1" test tube and 5 ml. water and 3 ml. concentrated H_2SO_4 are added. This is boiled until charring begins, and then 3 ml. selenium digestion mixture are added. The mixture is digested for several hours, cooled and diluted to 100 ml. in a volumetric flask.

The method employed from this point was the vacuum distillation technique of Rinehart, Grondahl and West (9). This digestion method and vacuum distillation was satisfactory will all of the compounds studied except copper sulfapyridine. For this compound the method of Klek and Sabotka (10) was employed. A weighed sample of the compound is placed in a 100 ml. volumetric flask with sufficient H_2SO_4 to affect solution. After diluting to volume a 10 ml. aliquot is placed in a 12" x 1" test tube with 80 mgs. HgO, 100 mgs. glucose, 1.0 gm. K_2SO_4, and 3 ml. concentrated H_2SO_4. This is heated cauticusly until foaming ceases, and the digestion completed in the usual way. The solution is diluted to 25 ml. and a 10 ml. aliquot taken for ammonia determination.

The same vacuum distillation apparatus is used but 0.75 gm. of sodium thiosulfate (11) is added with the alkali used to liberate the

*The sodium thiosulfate was standardized against pure copper as suggested by Bromund and Steiner (8).

ammonia. The object of the sodium thiosulfate is to convert the mercury used as a digestion catalyst to mercuric sulfide, preventing the formation of mercuric ammonium sulfate. The ammonia is then distilled into 4 per cent boric acid instead of into standard hydrochloric or sulfuric acid. The ammonium borate formed is titrated with standard acid to methyl red endpoint, and the amount of the nitrogen in the sample calculated.

The methods as described above were checked against standard solutions of the element to be determined. Satisfactory results were obtained in each case.

Analysis of sulfanilamide copper complex shows 11.1 per cent sulfur, 9.6 per cent nitrogen and 33.3 per cent copper, which indicates 3 atoms of copper, 4 atoms of nitrogen and 2 atoms of sulfur per molecule. The results are uniformly high, however, for a compound containing 2 molecules of sulfanilamide and 3 atoms of copper. There is no water of crystallization. The formula $(C_6H_8N_2SO_2)_2Cu_3(OH)_2$ is in good agreement with the analytical data.

Analysis of sulfathiazole copper complex shows 1 atom of copper, 3 atoms of nitrogen, and 2 atoms of sulfur per molecule which indicates 1 atom of copper per molecule of drug in the compound. The analytical data for sulfadiazene, monomethyl sulfadiazene, and dimethyl sulfadiazene, show the same ratio of drug to copper.

The following table gives the result of the analyses of several copper sulfonamide complexes. In each instance several determinations were made on at least two difference preparations of the compound.

TABLE VII

Sulfur, copper, and nitrogen content of various cuprous-copper sulfonamide complexes.

Copper Complex	Sulfur		Nitrogen		Copper	
	Found	Calculated*	Found	Calculated*	Found	Calculated*
Sulfanilamide	11.1	11.25	9.6	9.8	33.3	33.5
Sulfathiazole	20.0	19.9	13.1	13.2	20.0	20.16
Sulfapyridine	10.47	10.24	13.51	13.46	20.36	20.35
Sulfadiazene	10.22	10.22	17.67	17.87	20.21	20.24
Monomethyl sulfadiazene	9.73	9.81	17.05	17.12	18.91	19.13
Dimethyl sulfadiazene	9.35	9.36	16.28	16.42	12.35	18.47

*After this data were compiled, formulas were developed to fit the analytical data. From this, theoretical values for sulfur, nitrogen, and copper were calculated. These formulas are found in the following table.

TABLE VIII

Proposed formulas for some of the cuprous-copper sulfonamide complexes.

Copper Complex	Proposed Formula
Sulfanilamide	$(C_6H_8N_2SO_2)_2Cu_3(OH)_2$
Sulfathiazole	$(C_9H_9N_3S_2O_2)Cu$
Sulfapyridine	$(C_{11}H_{11}N_3SO_2)Cu$
Sulfadiazene	$(C_{10}H_{10}N_4SO_2)Cu$
Monomethyl sulfadiazene	$(C_{11}H_{13}N_4SO_2)Cu$
Dimethyl sulfadiazene	$(C_{12}H_{16}N_4SO_2)Cu$

Succinyl sulfathiazole, acetyl sulfanilamide and phthalyl sulfathiazole do not form cuprous-copper complexes under the conditions

employed. This led to the assumption that only sulfonamide drugs with a free para-amino group would form these complexes. An exception to this was recently observed, however, when a crystalline complex was formed with N^4 nicotinyl sulfanilamide under conditions used for the preparation of the other compounds. Thus the early interpretation of the mechanism of crystal formation was in error and at present the position of the cuprous ions and the valence arrangements of them in these compounds is unknown.

PART III

IDENTIFICATION OF SULFONAMIDE DRUGS BY THE COLOR AND MICROSCOPIC APPEARANCE OF THEIR COPPER COMPLEXES.

Since the copper sulfonamide complexes have characteristic crystalline habits, these are of value in the identification of the drugs.

For identification the crystals have been prepared as follows: To 5 ml. Shaffer-Hartmann reagent No. 50, 10 ml. water, 3 mgs. glucose and 30 mgs. of the sulfonamide drug are added. After 3 minutes in a boiling water bath the crystals form and can be identified by their characteristic shape.

Photomicrographs on Page 26 show six types of sulfonamide copper crystals prepared in the above manner.

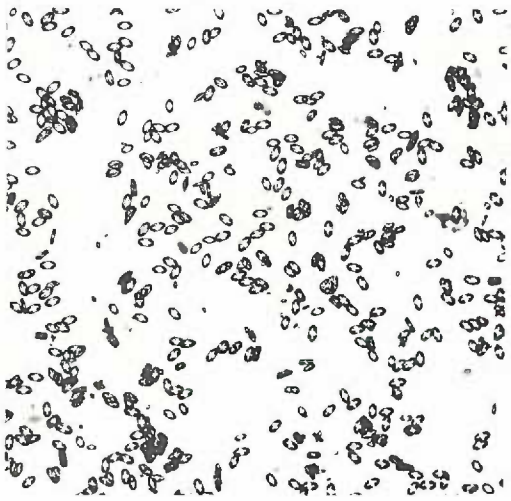
It is also an aid in identification to examine the color and gross appearance of the crystals. Sulfanilamide copper crystals do not precipitate until the solution is cooled and shaken. They form a sparse crop of fine, white granular crystals. Sulfathiazole copper complex is a loose, flocculent, white precipitate. Sulfapyridine copper complex is a granular, white precipitate. Sulfadiazene copper crystals are yellow and granular. Frequently cuprous oxide is formed with these crystals, but offers no interference in the microscopic examination. Monomethyl sulfadiazene copper crystals appear yellow and flocculent and dimethyl sulfadiazene copper compound crystallizes white and flocculent.

It is possible to take any one of these six sulfonamide drugs and identify it by this means in 5 to 10 minutes. It was hoped that this method of identification would be applicable to body fluids. To date it has been impossible to prepare characteristic crystal complexes from

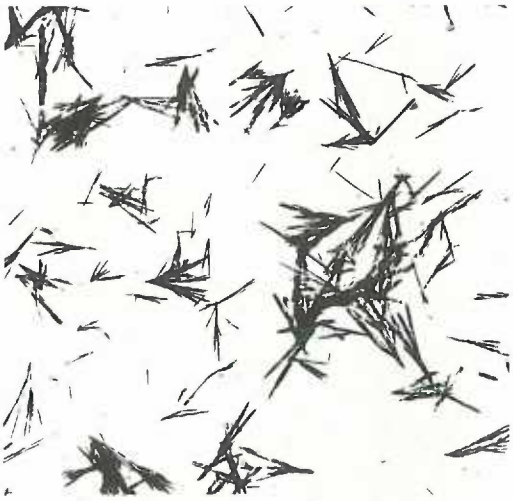
urine containing these drugs. The reason for this is not clear but it is possible that the many other substances present in urine prevent the reaction.

FIGURE I

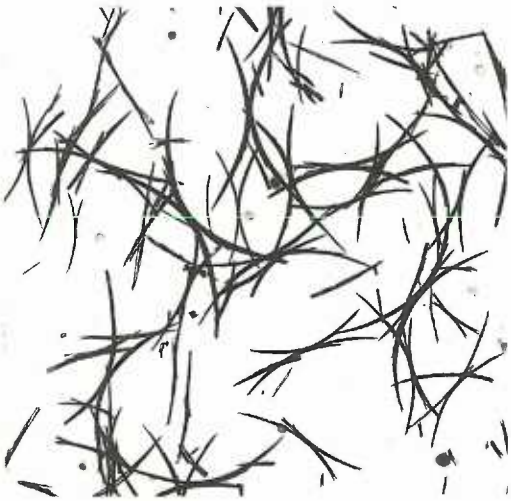
Cuprous-copper Sulfonamide Compounds



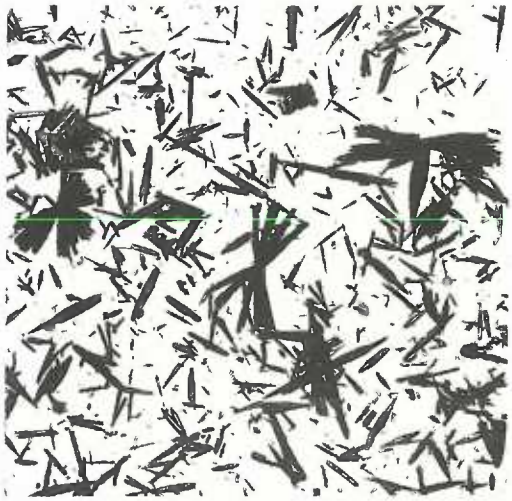
SULFANILAMIDE



SULFATHIAZOLE

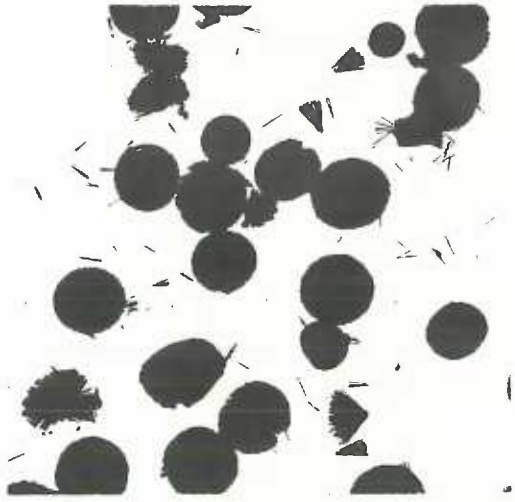


DIMETHYL SULFADIAZINE



SULFADIAZINE

SULFAPYRIDINE



MONOMETHYL SULFADIAZINE

FIGURE 1.
CUPROUS-COPPER SULFONAMIDE COMPOUNDS

SUMMARY

1. It has been demonstrated that sulfanilamide interferes in qualitative and quantitative sugar determinations when certain alkaline copper reagents are employed.

2. Data are presented indicating the degree of interference in quantitative determinations of sugar in blood and urine.

3. Because of the dilution factor involved in the quantitative tests, the error is not sufficient to be of clinical significance. The percentage error is dependent on the amount of glucose and sulfanilamide present.

4. The mechanism of sulfanilamide interference is due to the formation of a cuprous-copper sulfanilamide complex. This removes part of the cuprous ion. In the quantitative test part of this complex formed is decomposed on acidification, but there is always a small, constant amount of complex which is not decomposed. This allows less cuprous ion for reaction with iodine and thus the results are erroneously low.

5. In quantitative sugar determinations sulfathiazole and sulfapyridine cause an error in a direction opposite to that found in the case of sulfanilamide. These results are unexplained.

6. Data are also presented showing the interference of sulfanilamide in qualitative urine sugar determinations.

7. The error in the qualitative test is sufficient to be of clinical importance, particularly in the case of diabetic patients on sulfanilamide therapy.

8. In the qualitative tests the results are erroneously low because cuprous oxide is removed from the solution as the complex is formed.

The tannish-white color of the copper sulfanilamide complex also tends to lower the readings.

9. Sulfathiazole, sulfapyridine, sulfadiazene, monomethyl sulfadiazene, and dimethyl sulfadiazene do not cause a similar interference. The conditions of alkalinity and concentration of glucose and sulfonamide drug in these tests are apparently improper for the formation of these complexes.

10. Cuprous-copper sulfonamide complexes are prepared by heating the sulfonamide drug in an alkaline copper solution in the presence of a reducing agent such as glucose.

11. The analytical methods used to determine the sulfur, nitrogen, and copper content of these compounds are given in detail.

12. Formulas proposed from the analytical data are as follows:

Sulfanilamide complex $(C_6H_8N_2SO_2)_2Cu_3(OH)_2$

Sulfathiazole complex $(C_9H_9N_3S_2O_2)Cu$

Sulfapyridine complex $(C_{11}H_{11}N_3SO_2)Cu$

Sulfadiazene complex $(C_{10}H_{10}N_4SO_2)Cu$

Monomethyl sulfadiazene complex $(C_{11}H_{13}N_4SO_2)Cu$

Dimethyl sulfadiazene complex $(C_{12}H_{16}N_4SO_2)Cu$

13. A review of the literature revealed no previous report of such copper sulfonamide complexes.

14. A technique is described for the identification of six of the commonly employed sulfonamide drugs. This involves the examination of the color and crystal forms of the cuprous-copper complexes described. Photomicrographs are presented.

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